NEW TECHNOLOGY FOR ANALYZING ETHYLENE AND DETERMINING THE ONSET OF THE ETHYLENE CLIMACTERIC OF APPLES

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Ethylene is the fruit ripening hormone (4) and is responsible for development of ripening capacity of apples on or off the tree and during refrigerated and controlled atmosphere storage. General acceptance of this concept first formulated by Kidd and West (10,11) in England and extended by Hansen (9) has taken over forty years. Much of the technology now applied to controlled atmosphere storage of apples and pears is directed at attenuating the synthesis or action of ethylene (1,7,12,13,14 & 17). Markedly beneficial results from CA technology can generally be achieved only if the fruits are harvested and stored before the ethylene climacteric has commenced (2,6,15). It is important to be able to predict and measure fruit maturity at harvest with means that relate closely with the natural development of ripening capacity and storage potential. This would be helpful to make rational decisions about storage and marketing particular lots of apples.

Numerous studies have been conducted to predict (3,8,18,20) and confirm optimum harvest dates for apples according to the intended uses (2,8,15). It is a general observation that fruits become more responsive to initiation of ripening by ethylene as they mature on the tree. This is seen in the ethylene dose/response for apples (15) and in experiments with ethylene and propylene (16,18) treatments to measure fruits readiness to ripen.

Liu (15) observed an important relationship between the minimum treatment time with ethylene and the initiation of ethylene climacteric of McIntosh apples; the minimum treatment time shortened as the fruits matured on the tree. Dr. Earl Seeley[1] of Wenatchee, WA, observed a similar phenomenon with apples and pears in that the time required for harvested fruits to initiate autocatalytic ethylene production when several fruits were kept together in sealed containers shortened as fruits matured on the tree. It was reasoned that this occurred because ethylene produced by the more mature fruits stimulated ethylene production by the less mature fruits and more so as maturity progressed. And Seeley used this relationship to determine optimum harvest dates for storage for

[1] Personal communication

several varieties of apples and pears. This provided a practical guide to achieve maximum size, color and quality without compromising storage potential. We have employed the relationships observed by Liu and Seeley in developing a procedure for determining harvest date for CA apples by the induced climacteric method. The procedure is based on measuring ethylene and is becoming widely used since the development of a portable ethylene detector (6). Moreover, the procedure determines how maturation is progressing with time and thus provides a means to predict in advance by up to a week or 10 days when the fruits on the tree may begin the autogenous ethylene climacteric. A description of the procedure follows with some results discussed in relation to flesh firmness,starch index and internal ethylene measurements.

Sampling Apples for Maturity/Ripening Development

An estimate of the date fruits on the tree will enter the ethylene climacteric and begin to ripen can be obtained from the number of hours required for fruits to accumulate 0.5 ppm ethylene in sealed containers (Fig. 1). Begin sampling about 10 days to 2 weeks before the estimated starting harvest date for long term CA storage.

Place ca. 10 fruits (king blossom or most developed) with an envelope of hydrated spray lime (ca. 100 g.) in a 5 to 10 liter airtight container. Seal the container with a tight fitting cover which has a rubber stopper inserted for gas sampling. Three to five 10-fruit samples should be used per lot or orchard at each sampling date. Leave the containers of fruit at normal room temperature. Avoid sealing the sample containers in areas likely to be contaminated with fuel or engine exhaust fumes or cigarette smoke.

Remove 1 cc of air from the headspace of the container after the fruit has set for 6 to 24 hours. Inject the sample in a gas chromatograph capable of detecting .1 ppm of ethylene. Add 1 cc of room air to the container so the pressure inside stays equal to external pressure.

Continue daily sampling for 5 days or until 0.5 to 1.0 ppm of ethylene accumulates in the sealed container (whichever comes first). Note the number of hours of enclosure required for the apples to begin autocatalytic ethylene production and accumulate 0.5 ppm to 1.0 ppm in the sealed container. Examine fruits for moldy core or decay after finishing sampling. Discard data if fruits are decayed.

Repeat the procedure with fruits harvested from the same block about 3 to 5 days later again using three to five 10-fruit samples.

To calculate the harvest date for long term CA storage, multiply the number of hours required to accumulate 0.5 ppm ethylene in the sealed container times 0.125 days/hour. The product of this multiplication is the number of days from the time the fruits were placed in the containers to the harvest date.

Example: Fruits placed in containers 8 a.m. on September 10. 0.5 ppm ethylene measured 8 a.m. September 14. Calculations: 96 hours x 0.125 days/hour = 11.5 days. September 10 plus 11.5 days = September 22. Follow-up samples taken September 15 required 48 hours to accumulate 0.5 ppm. Calculations: 48 hours x 0.125 days/hours = 5.8 days. September 15 plus 5.8 days = September 21. Samples harvested September 21 should accumulate ethylene overnight if the projection was valid. Alternatively, the ethylene accumulation data can be plotted on graph paper to project the long term CA harvest date (Fig. 2).

The data in Table 1 indicates that samples collected as early as Sept. 17 were useful in predicting Oct. 3-5 as the date Empire apples left on the tree would enter the autogenous ethylene climacteric. Fruits harvested from these trees beginning the week of Oct. 2 stored well in controlled atmosphere storage with good retention of firmness and freedom from storage disorders. Flesh firmness at harvest did not indicate development of ripening potential whereas ripening capacity as measured by flesh softening in 7 days of harvested fruits had developed by Oct. 2. The starch index is also seen to progress gradually over the sampling interval of Sept. 24 - Oct. 2 while the hours to the induced ethylene climacteric (IEC) decreases markedly (128 to 24). Internal ethylene as measured by the mean or median also remains below 0.1 ppm over this interval with some fruits exceeding 0.1 ppm by Oct. 2. However, by Oct. 9 the mean and median ethylene levels are 0.97 and 0.2 ppm, respectively, and some fruits contain several ppm of ethylene.

Maturity indices for "MacSpur" in Table 2 also show that flesh firmness, starch index and internal ethylene do not change markedly over the period 8/29 - 9/9 while the hours to the induced ethylene climacteric decrease from 117 to 40. Moreover, even at the harvest of 9/9 the starch index is still very low indicating immature fruit and only changes from 2.2 to 2.7 during a 40-hour postharvest period at 20^{o}C. A harvest date of 9/10 - 9/15 was calculated from the IEC data and most generally was 9/14 - 9/15.

The induced ethylene climacteric procedure can also be useful to assess effectiveness of growth regulator treatments that may be used to hasten or delay fruit maturation. The effect of daminozide treatment to delay maturity of McIntosh is clearly evident in Table 3 for orchards A and C. A harvest date of 9/16 was predicted for orchard B from samples taken 8/28 and 9/11 but this was delayed to 9/20 - 9/22 based on samples taken 9/4, 9/11, and 9/18. A range of predicted harvest dates from 9/21 to 10/3 is shown for McIntosh orchards D, E, F, & G which typifies the variation found from orchard to orchard.

It is important to understand the role of ethylene in fruit ripening and its relationship to the change in starch index and flesh firmness as fruits develop on the tree. Starch content and flesh firmness per se are not unequivocal bench marks useful to define a particular stage of maturation and ripening. Starch begins to accumulate in a developing apple fruit 3 to 4 weeks after bloom after the period of cell division has been completed. Over the next 2 months it accumulates to a maximum value and subsequently declines as the fruits enlarge and mature. The pattern of starch accumulation and decline is a smooth bell-shaped curve depicting the balance of photosynthetic activity and metabolic demand. Absolute starch content does not have a fixed value chronologically speaking nor in relation to physiological development and maturation. Starch is a reserve carbohydrate that is eventually and quantitatively converted to soluble sugars metabolized by the cell in respiration. Consequently, the starch index of apples as developed by staining with a solution of I_2-KI will eventually depict this gradual disappearance of starch. Starch hydrolysis is not an ethylene/ripening-linked process apart from the stimulation of respiration rate by ethylene and the ensuing increase in carbohydrate catabolism. Consequently, apples may be stimulated to produce ethylene and begin to ripen without a visual change in the starch index. Alternatively, fruits may show depletion of starch while at pre- and postclimacteric stages with respect to ethylene.

The flesh firmness decrease observed as apples mature on the tree before ripening begins is likewise not an ethylene-linked process. The decrease in flesh firmness during maturation is the result of growth by cellular expansion and an increase in the intercellular air space. And, fruits may remain at a fixed flesh firmness value for a time while the biochemical capacity to ripen is developing without notice in response to ethylene action. Minimum flesh firmness values are helpful though as a criterion for durability for handling and storage and to assess condition and ripening development.

Late season biological, chemical, and environmental stresses may initiate or delay the onset of the ethylene climacteric and thus impact directly on the potential storability of apples. It therefore becomes important to be able to determine or estimate the time of the normal developmnt of the autogenous ethylene climacteric several days before the fact and confirm this as the season progress in order to make the appropriate decisions regarding disposition of the fruit.

Progress is being made by numerous researchers toward the important objective of predicting and determining optimum harvest dates of apples according to the handling and storage technology to be employed and the marketing period. Measuring ethylene levels in fruits and determining the fruits' propensity to initiate autocatalytic ethylene production and thus its capacity to ripen can be helpful in making this important assessment.

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TABLE 1. Prediction of harvest date for Empire apples based on induced ethylene climacteric in relation to other maturity indices.

Harvest Date	Flesh Firmness (lbs)		Starch Index	Internal Ethylene (ppm)			Hours* to IEC	Predicted Harvest Date**
	0	+1week		x	m	range		
9/17	16.2	16.6	1.0	0.044	.033	0.01-0.10	128	10/3
9/24	18.6	18.1	2.6	.035	.029	.01-0.07	91	10/5
9/30	17.0	16.6	3.2	.064	.074	.01- .10	43	10/5
10/2	18.1	16.2	3.7	.095	.097	.04- .16	24	10/3
10/9	17.4	15.6	4.5	.97	.22	.02-2.6		

* Average of 5 samples of 10 fruits each enclosed with dry lime. Hours to 0.5 ppm ethylene.

** Estimated from relationship:
(hours to IEC) x 0.125 days/hour = days to autogenous ethylene climacteric from sampling date.

TABLE 2. Maturity Indices of 'MacSpur'

Harvest Date	Starch Index	Flesh Firmness (lbs)	Median Internal Ethylene (ppm)	Hours to[1/] IEC	Predicted Harvest Date[2/]	Starch Index at IEC
8/29	2.1	15.6	0.052	117	9/14	2.5
9/2	1.4	16.4	0.044	68	9/10	2.5
9/5	1.8	15.8	0.028	77	9/15	2.7
9/9	2.2	15.5	0.039	40	9/14	2.7

1/ Hours to induced ethylene climacteric of harvested fruits.

2/ Estimated from relationship:
(hours to IEC) x 0.125 days/hour = days to autogenous ethylene climacteric from sampling date

TABLE 3. Prediction of autogenous ethylene climacteric of McIntosh by induced ethylene climacteric.

McIntosh Orchard	Sampling Date	Hours to IEC	Predicted Harvest Date
A. GS - Daminozide	9/7	78	9/17
	9/12	15	9/19
GS + Daminozide	9/7	168	9/28
	9/12	168	10/3
	9/17	107	9/30
B. HRC - Daminozide	8/28	148	9/16
	9/1	119	9/16
	9/4	140	9/22
	9/11	72	9/20
	9/18	30	9/22
C. GR + Daminozide	9/24	95	10/6
Daminozide + promalin	9/24	95	10/6
Daminozide + benzyladenine	9/24	88	10/5
Benzyladenine	9/24	22	9/25
Control	9/24	22	9/25
D.	9/12	168	10/3
E.	9/14	127	9/30
F.	9/16	38	9/21
G.	9/16	65	9/24

Figure 1.

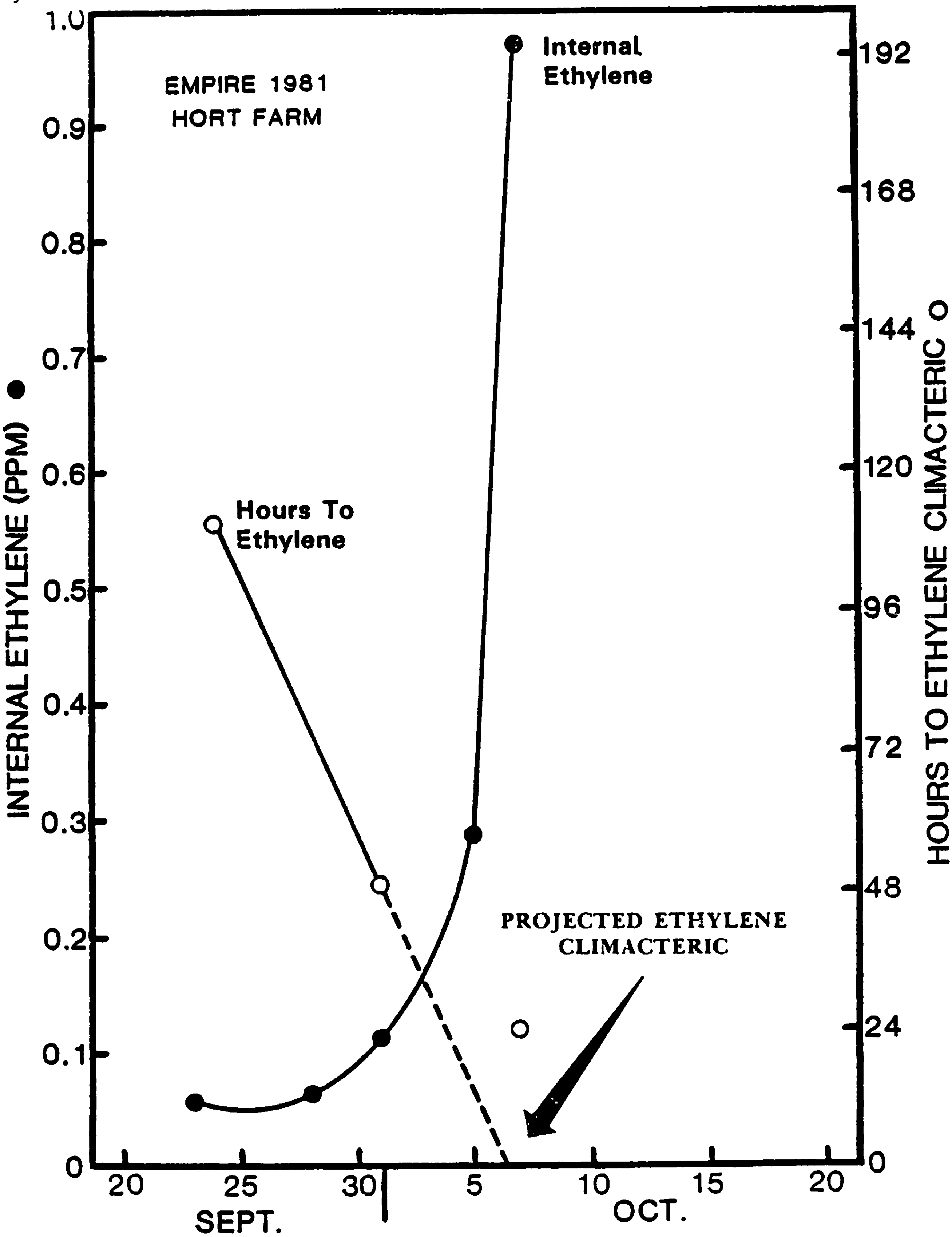
EMPIRE 1981
HORT FARM
Internal Ethylene
Hours To Ethylene
PROJECTED ETHYLENE CLIMACTERIC
INTERNAL ETHYLENE (PPM) ●
HOURS TO ETHYLENE CLIMACTERIC ○
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
192
168
144
120
96
72
48
24
0
20
25
30
5
10
15
20
SEPT.
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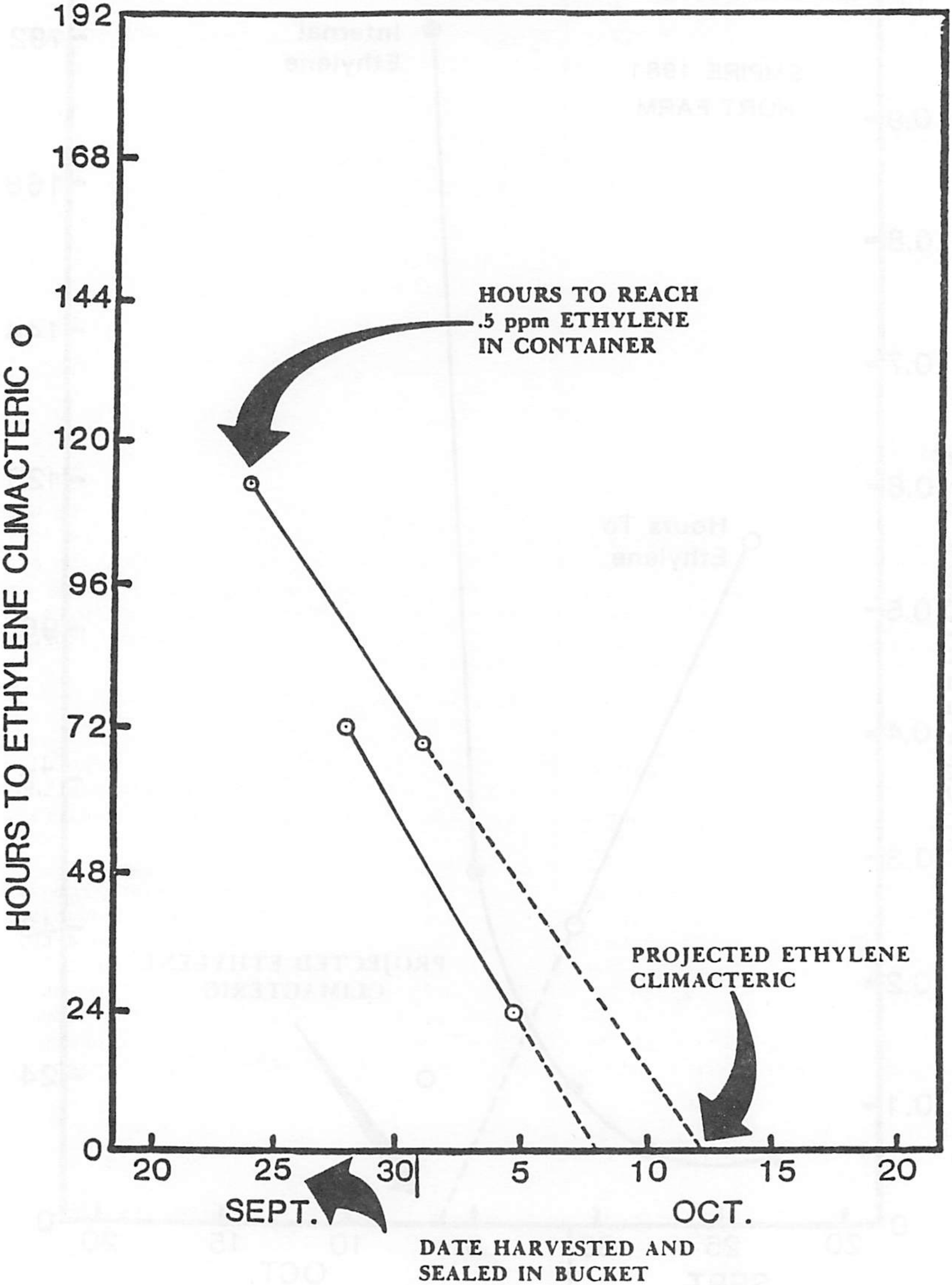
Figure 2.



HOURS TO ETHYLENE CLIMACTERIC O
192
168
144
120
96
72
48
24
0
HOURS TO REACH .5 ppm ETHYLENE IN CONTAINER
PROJECTED ETHYLENE CLIMACTERIC
20
25
30
5
10
15
20
SEPT.
OCT.
DATE HARVESTED AND SEALED IN BUCKET